Dietary Intake of Resveratrol Enhances the Adaptive Immunity of Aged Rats

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Abstract

It is well known that immune response declines with aging. Resveratrol, a polyphenol that occurs naturally in several plant species including grapevines and berries, has been shown to have potent antiaging and health-promoting activities. However, the mechanism underlying these activities remains largely unknown. Here we clearly demonstrate that: (1) Dietary intake of resveratrol induced a significant increase in T helper cells (CD4⁺) in middle-aged (12 months old) and aged (21 months old) Wistar male rats; (2) resveratrol supplementation considerably increased the delayed-type hypersensitivity response, a T cell-mediated immune response, in aged rats; and (3) resveratrol supplementation remarkably promoted the production of total anti-keyhole limpet hemocyanin (KLH) immunoglobulin G (IgG), anti-KLH IgG₁, and anti-KLH IgG_{2a} in aged rats without disturbing immune homeostasis. These data together indicate that resveratrol is capable of counteracting immunosenescence, thereby leading to rejuvenation. In practice, resveratrol can be useful to help the elderly generate an improved response to vaccine with stronger humoral and cell-mediated immune responses.

Introduction

AGING AFFECTS THE IMMUNE SYSTEM, leading to diminished overall functions. This phenomenon has been termed immunosenescence, which is clearly manifested by age-dependent defects in both humoral and cell-mediated immune responses.^{1–3} One of the best-characterized changes commonly observed in elderly subjects and old mice is the deficiencies in T cell functions that are exemplified by decreased T cell memory and exhaustion of the naïve T cell population with involution of the thymus.^{4,5} Among *in vivo* parameters of T cell-mediated immune response, the delayed-type hypersensitivity (DTH) reaction is depressed with aging. Particularly, low or no DTH responses are often predictive of morbidity and mortality.^{6–8} Although the B cell compartment of the immune system is only influenced to a minor extent by immunosenescence, antigen-specific responses to vaccination are altered with aging.⁹ As a result, elderly subjects are less able to mount an immune response after challenges with pathogens than are young adults and are more susceptible than the young to microbial infections, reactivation of latent viruses, autoimmune diseases, and neoplasia that contribute to morbidity and mortality.^{10–12}

It is well known that nutritional status exerts profound effects on immunity.¹³ Diet intervention with essential nutrients or functional foods are thus increasingly considered an effective approach to improve the immune functions.¹⁴ For example, dietary supplementation with nutrients with antioxidant properties, such as β -carotene, vitamin E, and lycopene, has been shown to improve immune function in aged mice and humans.^{15–17} Resveratrol (3,5,40-trihydroxystilbene), a polyphenol, is a bioactive substance with multiple functions that occurs naturally in several plant species, including grapevines and berries. Accumulating data have suggested that resveratrol has anticarcinogenic, antiinflammatory, antimicrobial, antiviral, and antioxidant properties that might be relevant to chronic diseases and/or longevity in humans.^{18,19} Resveratrol has also been found to be an effective neuroprotective compound.²⁰ More importantly, resveratrol has been claimed to possess antiaging activity. For example, resveratrol has been shown to be able to prolong the life span and retards the onset of age-related markers in a short-lived fish as well as in the invertebrates nematode worms and fruit flies.^{21–25} However, the mechanism underlying these observed antiaging effects is poorly understood. The present study was therefore undertaken to address this issue.

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Materials and Methods

Animals and treatments

All animal experiments were approved by the Ethics Committee of the Laboratory Animal Administration of Shandong province (permit number SD2007695). Specific pathogen-free male Wistar rats (*Rattus norvegicus*) aged 8 weeks (no. 20080002) were purchased from Shandong Lukang Pharmaceutical Co. Ltd and housed in an environmentally controlled atmosphere (temperature 22°C and relative humidity 56%) with a 12-hr light/dark cycle. Rats were given free access to water and diet (see below) and were provided with shredded wood floor bedding for social activity. They were housed 5 per cage until the age of 3 months and then housed 2 per cage. Wistar male rats have a life span of approximately 24–30 months; thus, 12-month-old and 21-month-old rats of this strain were used as a model of middle-aged and aged rats, respectively. Body weight was measured throughout the study period.

Both young (2 months old, $n=20$) and middle-aged (12 months old, $n=20$) as well as aged (21 months old, $n=20$) rats were randomly divided into two groups of 10 animals: (1) Rats of control groups ($n=10$ rats per age group) were fed with the controlled diet (complete semisynthetic columniformed diet containing 18% crude proteins and 5% cellulose following the Chinese Association For Laboratory Animal Sciences), and (2) rats of experimental groups (resveratrol group, $n=10$ rats per age group) were fed with the controlled diet supplemented with resveratrol at a dose of 22 mg/kg of body weight continuously for 45 days (Fig. 1). The dose of resveratrol used in this study was according to the observations in fish.²¹ Resveratrol was dissolved in distilled water (10 mg/mL). A portion of resveratrol solution (correlated with each rat body weight) was taken and injected into a small piece of the controlled diet. The food with injected resveratrol was given to the rat, making sure it ate the food completely. Drinking bottles with fresh mineral water were replaced daily. Throughout the 45-day study period, rats were allowed free access to diet and drinking water. Body weights were measured and recorded every 10 days. At the end of the study, rats were killed under anesthesia.

Chemicals

Resveratrol isolated from *Polygonum cuspidatum* Sieb et Zucc (purity >98%, No. 011-5) was donated by JF-Natural

(Tianjin, China). Keyhole limpet hemocyanin (KLH), fetal calf serum (FCS), concanavalin A (ConA), and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St. Louis, MO). Imject Alum was from Thermo Scientific (Rockford, IL). Biotin-conjugated goat anti-rat immunoglobulin G (IgG), and mouse anti-rat IgG₁ and IgG_{2c} were from Abcam (Cambridge, UK). Phycoerythrin (PE)-conjugated anti-rat CD3 antibody (T cells; clone eBioG4.18) was from eBioscience (San Diego, CA), and fluorescein isothiocyanate (FITC)-conjugated anti-rat CD4 (T helper cells; clone W3/25), PE-conjugated anti-rat CD8 (T cytotoxic cells; clone G28), and FITC-conjugated anti-rat CD44 (memory T cells; clone OX-49) antibodies were from Biolegend (San Diego, CA). CellTiter 96 AQueous One Solution Reagent was from Promega (Madison, WI). 3,3',5,5'-tetramethylbenzidine peroxidase substrate was from Sangon (Shanghai, China). Penicillin and streptomycin were from Klontech (Jinan, China), HEPES was from Solarbio (Beijing, China), and 96-well plates were from Corning Incorporated (Corning, NY).

Immunization protocol

Rats were immunized as described by Vidal et al.²⁶ The T cell-dependent humoral response (antigen-specific antibody production) was measured after immunization of rats with KLH, an innocuous protein isolate that generates a strong T cell-mediated antibody response and has been used extensively in animals. All rats were immunized on day 15 of the study with a subcutaneous injection of 250 μ g of KLH emulsified in 1% Imject Alum. Blood samples were collected on days 0, 15, 30, and 45 for serum or plasma preparation (Fig. 1).

DTH response assay

The DTH response was used as an *in vivo* measure of cellular immunity. The response was calculated as the difference in ear thickness of the rats before and after challenge with a recall antigen. DTH response assay was performed according to the method of Vidal et al.²⁶ In brief, on day 22 of the study (*i.e.*, 7 days after KLH immunization), the thickness of the ears was measured with a micrometer, and immediately thereafter the DTH response was elicited by intradermal injection of KLH (20 μ L of 1 μ g/mL in phosphate-buffered saline [PBS]) into the right ear. For control, PBS (20 μ L) alone was

FIG. 1. (A) A schematic diagram of the experiment. An asterisk (*) indicates the time points at which the different trials were carried out. Rats of control and experimental groups were fed with controlled diet or diet supplemented with resveratrol, respectively. Body weights were measured on 0, 10, 20, 30, and 40 days of the trial. Blood was sampled on 0, 15, 30, and 45 days of the trial. Delayed-type hypersensitivity (DTH) response was assayed on day 22 of the trial (*i.e.*, 7 days after keyhole limpet hemocyanin [KLH] immunization). Spleens were collected on 45 day of the trial (*i.e.*, sacrifice day). RSV, Resveratrol.

injected into the left ear. At 12 h and 24 h after challenge, the thicknesses of the control ear (PBS) and the challenged ear (KLH) were measured. The DTH responses were expressed as the magnitude of ear swelling, using the following formula: Δ in ear thickness of challenged ear (KLH) – Δ in ear thickness of control ear (PBS), where Δ in ear thickness = ear thickness postchallenge – ear thickness prechallenge.

Blood sampling and hematologic index/biochemical parameter assays

Blood samples were collected from the tail vein on days 0, 15, and 30 of the study. At sacrifice (day 45), blood was collected from living heart. Blood samples were divided into two parts: One part of the blood was directly used for hematologic index assay, and the other part was used to prepare serum by centrifugation at $10,000 \times g$ at 4°C for 15 min. The hematologic indexes of the blood, including red blood cells (RBCs), white blood cells (WBCs), hemoglobin (HGB), and platelets (PLT), were analyzed by an automatic hematologic analyzer (Sysmex XS-800i). The biochemical parameters of the serum, including glutamic pyruvic transaminase (ALT), aspartate transferase (AST), triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL), were analyzed immediately using an automatic biochemistry analyzer (Hitachi P7600). The remaining serum was aliquoted and stored at -80°C until used.

KLH-specific IgG concentration assay

The levels of KLH-specific IgG, IgG₁, and IgG_{2x} in the blood were measured by an enzyme-linked immunosorbent assay (ELISA) according to the method of Vidal et al.²⁶ with a slight modification. Briefly, microtiter plates were coated with KLH ($50 \mu\text{L}/\text{well}$ at $5 \mu\text{g}/\text{mL}$ for total IgG and IgG₁ and $10 \mu\text{g}/\text{mL}$ for IgG_{2x}), incubated at 37°C for 2 hr and blocked with 5% FCS for 1 hr at 37°C . Serum samples were then added into the wells after dilution ($1/2,000$ for total IgG, and $1/100$ for IgG₁ and IgG_{2x}), and the plates were incubated at 37°C for 1 hr. Bound antibodies were detected following incubation with a biotin-conjugated goat anti-rat IgG ($1/200,000$) or mouse anti-rat IgG₁ ($1/20,000$) and IgG_{2x} ($1/20,000$) at 37°C for 1 hr in a water bath. Plates were read at 450 nm after the addition of the TMB peroxidase substrate. Anti-KLH IgG, IgG₁, and IgG_{2x} were expressed as median of normalized optical density (O.D.) at 450 nm.

Assay for cellular composition of splenocytes

The percentages of major T and B cell subsets in spleen were assayed using fluorescence-activated cell sorting (FACS) analysis. Single-cell suspensions of splenocytes (1×10^6 cells/sample) were stained for cell-surface antigen expression with following antibodies: PE-conjugated anti-rat CD3, FITC-conjugated anti-rat CD4, PE-conjugated anti-rat CD8, and FITC-conjugated anti-rat CD44. Unlabeled cells were used as a negative control. Cells were analyzed on a Beckman EPICS XL-MCL ADC flow cytometry equipped with System IITM analysis software.

FIG. 2. Body weights of young (2 months old; A), middle-aged (12 months old; B), and aged (21 months old; C) rats fed with controlled diet or diet supplemented with resveratrol for 45 days. Values are medians of kilograms $\pm$ standard error (SE) of medians. RSV, Resveratrol.

Assay for proliferation of splenocytes

Splenocytes were suspended in complete RPMI-1640 medium containing 100 U/mL penicillin, 100 mg/mL streptomycin, 10 mM HEPES, and 10% heat-inactivated FCS. Cell viability was tested by Trypan Blue exclusion. Splenocytes

(1×10^5 cells/well) were seeded in 96-well plates and cultured in the presence or absence of the T cell mitogen ConA at $10 \mu\text{g/mL}$, or the B cell mitogen LPS at $5 \mu\text{g/mL}$ or the antigen KLH at $100 \mu\text{g/mL}$ at 37°C for 64 hr in an atmosphere of 5% CO_2 and 95% humidity. Subsequently, $20 \mu\text{L}$ of CellTiter 96 AQueous One Solution Reagent was added into each well and incubated at 37°C for 4 hr in a humidified atmosphere with 5% CO_2 . The absorbance at 490 nm was measured under a spectrophotometer (Tecan, Austria).

Statistical analysis

Statistical analysis was performed using the Student *t*-test in the software SPSS 13.0. Analysis was done separately for the young rat study, the middle-aged rat study, and the aged rat study because these studies were carried out independently. Data were expressed as the median $\pm$ standard error (SE) of the median. Probability values of less than 0.05 were considered as significant.

Results

General condition and physiology of rats

Young, middle-aged, and aged male rats in all diet groups looked healthy throughout the experiment. Body weights were not significantly affected by dietary resveratrol supplementation. As shown in Fig. 2, median body weight values of young, middle-aged, and aged rats were not different among the diet groups at the start of the study and increased from 0.31 ± 0.01 to 0.49 ± 0.12 kg in young rats and from 0.56 ± 0.02 to 0.62 ± 0.02 kg in middle-aged rats (Fig. 2A,B). Median body weight values of aged rats were 0.64 ± 0.02 kg at the beginning of the study (day 0), which remained unchanged until the end of the trial (Fig. 2C). In addition, median hematological indices (see Table S1; Supplementary data are available at www.liebertonline.com/rev/) and biochemical parameters including ALT/AST activities and TG, TC, HDL, and LDL levels (Table S2) of young, middle-aged, and aged rats all remained similar among the diet groups, suggesting that the general physiology of the rats was not affected by dietary resveratrol supplementation. These data indicated that no significant differences in general condition and physiology were observed among the diet groups in young, middle-aged, and aged male rats throughout the trial period.

Effects of resveratrol on cellular composition of splenocytes *in vivo*

As shown in Table 1, resveratrol supplementation did not affect the percentage of total T cells (CD3^+), T helper cells (CD4^+), T cytotoxic cells (CD8^+), and memory T cells (CD44^+) in spleen from young male rats. Notably, resveratrol supplementation caused a significant increase in the percentage of T helper cells (CD4^+) in spleen from middle-aged (22.23 ± 3.74 , $p = 0.019$) and aged (22.27 ± 4.39 , $p = 0.040$) rats compared to control groups (17.53 ± 3.66 for middle-aged rats and 17.64 ± 4.08 for aged rats) (Fig. 3). Resveratrol supplementation had little influence on the percentage of total T cells (CD3^+), T cytotoxic cells (CD8^+), and memory T cells (CD44^+) in spleen from middle-aged and aged rats (Fig. S1). Clearly, T helper cells (CD4^+) *in vivo* were markedly increased in middle-aged and aged rats by dietary resveratrol supplementation.

Effects of resveratrol on *ex vivo* proliferation of splenocytes

Table 2 shows the *ex vivo* proliferative response of rat splenocytes to resveratrol. Upon stimulation with ConA, a T cell mitogen, or with LPS, a B cell mitogen, the proliferative response of splenocytes from young rats fed resveratrol-supplemented diet was considerably increased compared with those fed the controlled diet. In contrast, dietary resveratrol supplementation had little effect on the proliferation of splenocytes from middle-aged and aged rats, upon stimulation with ConA or LPS. Young rats fed resveratrol-supplemented diet exhibited a significantly higher proliferative response upon stimulation with the immunogen KLH than those fed the controlled diet, whereas such an effect was not observed in middle-aged and aged rats (Fig. S2). These results showed that no age-associated difference in ConA-, LPS-, and KLH-stimulated proliferation was found in splenocytes from middle-aged and aged rats.

Effects of resveratrol on antigen-specific humoral and cell-mediated immune responses

KLH-specific serum $\text{IgG}_{2\alpha}$ and IgG_1 levels, measured after immunization with KLH, were used as markers of T helper 1 (Th1)- and Th2-dependent humoral responses, respectively. Resveratrol supplementation did not significantly affect total serum IgG levels in young, middle-aged, and aged

TABLE 1. CELLULAR COMPOSITION OF SPLENOCYTES FROM YOUNG, MIDDLE-AGED, AND AGED RATS FED A CONTROLLED DIET OR A DIET SUPPLEMENTED WITH RESVERATROL ASSAYED BY FLOW CYTOMETRY^{a,b}

Cells/makers	Young rats			Middle-aged rats			Aged rats		
	C (n=10)	Resveratrol (n=10)	p	C (n=10)	Resveratrol (n=10)	p	C (n=10)	Resveratrol (n=10)	p
CD3^+	41.89 ± 4.17	42.55 ± 7.19	NS	40.24 ± 7.23	40.89 ± 6.45	NS	39.99 ± 7.19	41.38 ± 5.78	NS
CD4^+	21.26 ± 4.12	19.29 ± 2.86	NS	17.53 ± 3.66	22.23 ± 3.74	0.019	17.64 ± 4.08	22.27 ± 4.39	0.040
CD8^+	21.21 ± 3.43	23.32 ± 7.41	NS	22.73 ± 7.23	18.24 ± 6.71	NS	23.26 ± 7.10	17.80 ± 5.57	NS
CD44^+	40.79 ± 7.85	41.66 ± 7.14	NS	39.31 ± 7.50	40.22 ± 6.04	NS	39.15 ± 6.71	40.36 ± 5.57	NS

^aThe percentages of major T and B cell subsets in spleen were assayed as described in Materials and Methods.

^bValues are medians of value $\pm$ standard error (SE) of medians.

C, Control; NS, not significant.

FIG. 3. Percentage of CD4⁺ T cells in the spleen from young (A), middle-aged (B), and aged (C) rats fed controlled diet or diet supplemented with resveratrol. The data of each individual animal are presented. RSV, Resveratrol.

rats (Table S3). As shown in Fig. 4, levels of total antigen-specific IgG (*i.e.*, anti-KLH IgG) and anti-KLH IgG_{2x} were both significantly increased in aged rats fed resveratrol-supplemented diet (Fig. 4C,I), but not in young and middle-aged rats (Fig. 4A,B,G,H). Similarly, the anti-KLH IgG₁

level was also remarkably increased in aged rats fed resveratrol-supplemented diet (Fig. 4F), although it was also markedly increased in young rats (Fig. 4D) by resveratrol supplementation. These results indicated that dietary resveratrol supplementation was able to induce a significant increase in total antigen-specific IgG, IgG₁, and IgG_{2x} levels in aged rats.

The DTH response was used to evaluate effects on cellular Th1-dependent immunity. Dietary resveratrol supplementation significantly affected the DTH response in aged rats at 24 hr and 48 hr after KLH challenge (Fig. 5). No difference in the DTH response was found in young and middle-aged rats fed resveratrol diet compared with the control group (Table 3; Fig. S3).

Discussion

Resveratrol is a multifunctional bioactive substance that has been shown to have anticarcinogenic, anti-inflammatory, antimicrobial, antiviral, antioxidant, and antiaging properties. This study investigated the effects of resveratrol on *ex vivo* and *in vivo* parameters of adaptive immunity in both young and middle-aged as well as aged rats. Dietary supplementation with resveratrol was started 2 weeks before immunization of the rats with a T cell-dependent antigen, KLH, and continued until the end of the experiment, so that the diet had the opportunity to modulate cell-mediated immune processes during both the initiation and promotion of the immune response toward the antigenic challenge. The data of our study apparently support the belief that resveratrol has antiaging effects by counteracting immunosenescence, which is mostly characterized by poor T cell-mediated immune function and low protection after vaccination. First, resveratrol induced a considerable increase in T helper cells (CD4⁺) in aged rats. Second, dietary resveratrol supplementation significantly increased the DTH response, a T cell-mediated immune response, in aged rats. Third, resveratrol supplementation remarkably promoted the production of total antigen-specific IgG, IgG₁, and IgG_{2x} levels in aged rats without disturbing immune homeostasis.

Incompetence to proliferate in response to antigen is typically a characteristic of the decline in T cell function with aging.²⁷ Here we showed that T helper cells (CD4⁺) in aged rats were significantly increased by dietary resveratrol supplementation. Moreover, although a marked increase in proliferative response of splenocytes to the recall antigen KLH was only observed in young rats fed a resveratrol-supplemented diet, splenocytes from young, middle-aged, and aged rats all proliferated in the presence of KLH. These results provided *ex vivo* evidence showing that a T cell-mediated memory response occurred. Dietary resveratrol supplementation significantly stimulated splenocyte proliferation to the T cell mitogen ConA and B cell mitogen LPS in young rats, although it had little effect on splenocyte proliferation in response to ConA and LPS in middle-aged and aged rats. This absence of splenocyte proliferation-stimulating effect by resveratrol supplementation in middle-aged and aged rats may be due to the mitogen concentrations used. Indeed, it has been shown that an increased proliferative response of splenocytes following supplementation with conjugated linoleic acid was observed only with suboptimal and supraoptimal

TABLE 2. PROLIFERATIVE RESPONSES OF SPLENCYTES FROM YOUNG, MIDDLE-AGED, AND AGED RATS FED A CONTROLLED DIET OR A DIET SUPPLEMENTED WITH RESVERATROL^{a,b}

Immunogens	Young rats			Middle-aged rats			Aged rats		
	C (n=10)	Resveratrol (n=10)	p	C (n=10)	Resveratrol (n=10)	p	C (n=10)	Resveratrol (n=10)	p
ConA (T cell mitogen)	0.46±0.06	0.64±0.01	0.00017	0.45±0.02	0.48±0.03	NS	0.42±0.02	0.43±0.03	NS
LPS (B cell mitogen)	0.49±0.01	0.60±0.02	0.006	0.48±0.04	0.50±0.01	NS	0.43±0.03	0.42±0.02	NS
KLH (antigen specific)	0.53±0.01	0.59±0.03	0.03	0.46±0.04	0.51±0.03	NS	0.41±0.04	0.41±0.02	NS

^aSpleocytes were cultured in the presence or absence of concanavalin A (ConA; 10 µg/mL), lipopolysaccharide (LPS; 5 µg/mL), or keyhole limpet hemocyanin (KLH; 100 µg/mL) for 64 hr and pulsed with CellTiter 96 AQueous One Solution Reagent during the last 4 hr. The optical density (O.D.) value was measured to determine the proliferative responses of cells.

^bValues are medians of OD value±standard error (SE) of medians.

C, Control; NS, not significant.

concentrations of ConA in young mice and optimal concentration of ConA in aged mice.²⁸

DTH response is an *in vivo* indicator of the ability to mount a T cell-mediated response. DTH is mainly driven by memory CD4⁺ T cells that release interleukin-2 (IL-2)

and interferon-γ (IFN-γ) to signal macrophages to an area after an encounter with the recall antigen. It has been widely used in nutrition immunology studies of elderly subjects and old mice.^{29–32} An age-associated difference in DTH response was noted in the presence of resveratrol.

FIG. 4. Anti-KLH IgG isotype (A–C), anti-KLH IgG₁ (D–F) isotype, and anti-KLH IgG_{2α} isotype (G–I) responses in young, middle-aged, and aged rats fed controlled diet or diet supplemented with resveratrol. Antibody levels were determined as described in Materials and Methods. Values are medians of measured optical density (O.D.) at 450 nm ± standard error (SE) of the median. The symbols ** ($p < 0.01$) and * ($p < 0.05$) indicate the differences of comparing resveratrol group to control group. RSV, Resveratrol; KLH, keyhole limpet hemocyanin; IgG, immunoglobulin G.

FIG. 5. Delayed-type hypersensitivity (DTH) responses of aged rats fed controlled diet or diet supplemented with resveratrol. The data of each individual animal are presented. RSV, Resveratrol.

Dietary resveratrol supplementation had little effect on the DTH response in young and middle-aged rats, but it significantly increased the DTH response in aged rats. These results suggest that T cell-mediated immune responses are benefited by dietary resveratrol supplementation. In practice, this may have a critical implication,

because DTH responses that decrease with age are inversely correlated with morbidity and mortality in elderly subjects.^{33,34}

Humoral response to antigen challenge is altered with aging.³⁵ For example, the primary antibody response to the KLH antigen has been reported to decline in elderly subjects.³⁶ An age-associated difference in T cell-mediated antibody response to the KLH antigen was also noted in the presence of resveratrol. Although resveratrol supplementation induced a significant increase in the anti-KLH IgG₁ level in young rats, it did not affect the production of total anti-KLH IgG, anti-KLH IgG₁, and anti-KLH IgG_{2 α} in middle-aged rats. In sharp contrast, dietary supplementation with resveratrol significantly promoted the production of total anti-KLH IgG, anti-KLH IgG₁, and anti-KLH IgG_{2 α} in aged rats. These observed changes in antibody responses in aged rats fed a resveratrol-supplemented diet are indicative of improvements in antigen-driven responses because total antigen nonspecific IgG levels were not affected. These results suggest that dietary resveratrol supplementation is able to modulate immune responses without disrupting immune homeostasis. Importantly, resveratrol supplementation led to a considerable increase in the KLH-specific IgG₁ antibody response in aged rats as well as a considerable increase in the KLH-specific IgG_{2 α} response in aged rats. It is known that the production of IgG_{2 α} is promoted by Th1 cytokines and the production of IgG₁ antibody by Th2 cytokines.³⁷ Thus, this observation suggests that diet with resveratrol supplementation could benefit both Th1- and Th2-type immune responses.

In summary, this study highlights the antiaging and immunostimulatory properties of resveratrol via enhancing *in vivo* humoral and cell-mediated immune responses in aged rats after antigen challenge. It also suggests that dietary intake of resveratrol may help the elderly to generate an improved response to vaccines with stronger humoral and cell-mediated immune responses.

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TABLE 3. DELAYED-TYPE HYPERSENSITIVITY RESPONSES OF YOUNG, MIDDLE-AGED, AND AGED RATS FED A CONTROLLED DIET OR A DIET SUPPLEMENTED WITH RESVERATROL^{a,b}

Time (hr)	Young rats			Middle-aged rats			Aged rats		
	C (n=10)	Resveratrol (n=10)	p	C (n=10)	Resveratrol (n=10)	p	C (n=10)	Resveratrol (n=10)	p
24	122±51	143±27	NS	220±46	236±41	NS	51±21	185±48	0.007
48	151±40	184±67	NS	112±25	209±58	NS	75±19	228±26	0.0009

^aTo elicit the delayed-type hypersensitivity (DTH) response, young, middle-aged, and aged rats were immunized and challenged with keyhole limpet hemocyanin (KLH) as described in Materials and Methods. DTH responses were measured as the difference in ear thickness and expressed in micrometers.

^bValues are medians±standard error (SE) of medians.

C, Control; NS, not significant.

Author Disclosure Statement

No competing financial interests exist. This work was conducted under the approval of JF-Natural and the Ethics Committee of the Laboratory Animal Administration of Shandong province. Shicui Zhang designed the research and wrote the manuscript; Jiangshui Yuan and Linlin Lu performed the research, analyzed the data, and wrote the manuscript; Zongliang Zhang contributed to data analysis and proofreading.

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